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nrDNA internal transcribed spacer data reveal that *Rhodoplagiochila* R.M. Schust. (Marchantiophyta: Jungermanniales) is a member of *Plagiochila* sect. *Arrectae* Carl

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Abstract

The monospecific genus *Rhodoplagiochila*, previously known only from the type collected in the Venezuelan Andes, has been collected at a second site close to the type locality. Up to now, generic status of *Rhodoplagiochila* has been accepted by most authors, but the taxon had been alternately assigned to Plagiochilaceae or Lophoziaceae. Phylogenetic analyses of nrITS sequences from *Rhodoplagiochila* and other representatives of the Plagiochilaceae clearly reveal the former to be in a clade with members of the Neotropical–Atlantic European *Plagiochila* sect. *Arrectae*. Within the *Arrectae*, *Rhodoplagiochila* is positioned in a robust clade made up of several accessions of *Plagiochila bifaria*. Morphologically, *Rhodoplagiochila* differs from other phenotypes of *P. bifaria* by the papillose leaf surface and a leaf margin which is ciliately toothed all-around. As an outcome of the molecular and morphological investigations, *Rhodoplagiochila rosea* is treated as a variety of *P. bifaria*, and the new combination *P. bifaria* var. *rosea* comb. et stat. nov. is proposed. In tropical America the family Plagiochilaceae seems to be represented only by *Plagiochila*.

Keywords: Plagiochilaceae; *Rhodoplagiochila*; *Plagiochila* sect. *Arrectae*; *Plagiochila bifaria*; Molecular phylogeny; ITS

Introduction

The cosmopolitan genus *Plagiochila* (Dumort.) Dumort. is the largest genus of hepatics, with about 1600 published binomials (Inoue 1989) and an estimated 400–450 morphologically defined species (So and Grolle 2000). Representatives of *Plagiochila* are able to form large mats, and are among the most diverse and abundant cryptogams in humid tropical forests. Large numbers of species also occur in temperate and arctic regions with oceanic climate.

Taxonomy at species and section level is hampered by the scarcity of stable morphological characters. The genus itself can easily be identified by its laterally compressed perianth with a dorsal keel usually longer than the ventral one, by dioicism, nearly exclusively lateral branching, succubous leaves, and alternating foliation. Several small or monospecific genera—e.g., *Chiastocaulon* Carl, *Pedinophyllum* (Lindb.) Lindb., *Plagiochilion* S.Hatt., *Rhodoplagiochila* R.M.Schust., *Steereochila* Inoue, *Szweykowskia* Gradst. and M.Reiner—have been separated from *Plagiochila* due to differences in one or two morphological characters.

Within the Neotropics, Plagiochilaceae are represented by numerous species of *Plagiochila*, as well as by

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the three monospecific genera *Rhodoplagiochila*, *Steereochila* and *Szweykowskia*. References to *Plagiochilion* are doubtful (see Heinrichs 2002).

Steereochila was set up because of its unusual type of vegetative reproduction by entire, thread-like propagules, and the occurrence of ventral–terminal branches (Inoue 1987). *Szweykowskia* was separated from *Plagiochila* because of its unique, saccate leaves and the supposedly ventral–intercalary branches (Gradstein and Reiner-Drehwald 1995). However, morphological reinvestigation of sporophytic and gametophytic characters of both genera indicated close relationships to several sections of *Plagiochila* (Heinrichs et al. 2001). Subsequent cladistic analyses of morphological and phytochemical characters as well as of nrITS sequence variation clearly revealed both taxa as members of the Neotropical–African *Plagiochila* sect. *Hylacoetes* Carl (Heinrichs 2002).

Rhodoplagiochila with the single species *Rhodoplagiochila rosea* R.M.Schust. was established because of the rose-red pigmentation of its shoot apices (Schuster 1978) which, however, disappears in herbarium material (Schuster 2000). Originally placed in Plagiochilaceae by Schuster (1978), the genus was transferred to Lophoziaceae by Inoue (1984). However, Schuster (2000) depicted the plagiochilid perianths of *Rhodoplagiochila* and provided convincing evidence that *Rhodoplagiochila* should remain within the Plagiochilaceae. Heinrichs (2002) doubted the generic status of *Rhodoplagiochila* and assumed this taxon to be a member of the Neotropical–Atlantic European *Plagiochila* sect. *Arrectae* Carl.

For a long time, *Rhodoplagiochila* was known only from the type collected in the high Andes of Mérida, Venezuela. Recently, a second locality was detected in the same region. The new specimens allowed to conduct a molecular-phylogenetic of *Rhodoplagiochila*.

Material and methods

Molecular investigation

DNA extraction

The upper parts of a few shoots of air-dried specimens were isolated. Genomic DNA was extracted with Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany).

PCR amplification and sequencing

nrITS: The 5′-primers Hep2f (5′-GAG TCA TCA GCT CGC GTT GAC-3′) and Hep3f (5′-CGG TTC GCC GCC GGT GAC G-3′), and the 3′ primers HepCr (5′-TCT CCA GAC TAC AAT TCG CAC A-3′) and HepAr (5′-CGC CGC TAC TAC GGA AAT CCT A-3′) (Groth et al. 2003) were used to amplify the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, containing ITS1, ITS2 and the 5.8S gene. Polymerase chain reaction (PCR) was performed in a total volume of 50 µl, containing one unit Taq-DNA-polymerase (SilverStar, EuroGenTech), 5 µl Taq polymerase reaction buffer (EuroGenTech), 2 µl $MgCl_2$ (50 mM), 1 µl dNTP-mix (10 mM, MBI Fermentas), 2 µl dimethylsulfoxid, 1 µl of both forward and reverse primer (10 mM), and 1 µl template. PCR was carried out using the following program: 120 s initial denaturation at 92°C, followed by 30 cycles of 60 s denaturation at 92°C, 50 s annealing at 51°C, and 90 s elongation at 72°C. Final elongation was carried out in one step (10 min 72°C). For the first PCR, the primers Hep2f-HepCr were used. If no product could be detected (detection on 1.5% agarose gel, stained with GelStar; MFC-Bioproducts), a second (nested) PCR was performed, using primers Hep3f-HepAr.

Sequencing was carried out on an ABI 3100 capillary sequencer using the BigDye™ Terminator Cycle Sequencing v2.0 kit (PE Biosystems).

Taxon sampling and phylogenetic analyses

The *Rhodoplagiochila* nrITS sequence was compared with GenBank sequences using the BLASTN program, and put into a large alignment of Plagiochilaceae ITS sequences with *Herbertus* and *Lophocolea* as outgroups. In both cases sequences of *Plagiochila* sect. *Arrectae* were identified as most similar to the new sequence (data not shown). Based on these results, representatives were sampled from eight Neotropical *Plagiochila* sections established in earlier analyses of ITS sequence variation (Groth et al. 2002, 2003; Heinrichs 2002; Heinrichs et al. 2002a, b, 2003, 2004; Rycroft et al. 2002), including several species of *Plagiochila* sect. *Arrectae*, *Plagiochila* (*Steereochila*) *dimorpha*, and *Plagiochila* (*Szweykowskia*) *cucullifolia*. The matrix was completed with *Rhodoplagiochila*. *Pedinophyllum interruptum* (Nees) Kaal. and *Plagiochilion mayebarae* S.Hatt. were chosen as outgroups, according to the results of Groth and Heinrichs (2003).

The 40 ITS1, 5.8S and ITS2 sequences (Table 1) were aligned manually in BioEdit version 5.0.9 (Hall 1999), resulting in an alignment including 729 putatively homologous sites (alignment available from JH).

Phylogenetic trees were inferred using maximum likelihood (ML) and maximum parsimony (MP) criteria as implemented in PAUP* version 4.0b10 (Swofford 2003).

ML analyses: To choose the nucleotide substitution model with the smallest number of parameters that best fits the data, the program Modeltest 3.06 (Posada and Crandall 1998) was used that employs two statistics: the likelihood ratio test (LRT) and the Akaike information criterion (Akaike 1974). Based on the results of the tests,

Table 1. Geographic origins, voucher numbers, and GenBank/EMBL accession numbers (bold for new sequences) of the taxa investigated. Vouchers are deposited at GOET unless indicated otherwise.

Taxon	Origin	Voucher	Accession number
Pedinophyllum interruptum (Nees) Kaal.	British Isles	*Rycroft 020907*	**AY438234**
Plagiochila adiantoides (Sw.) Lindenb.	Costa Rica	*Heinrichs et al. 4314*	AJ422027
P. aerea Taylor	Costa Rica	*Heinrichs et al. 4321*	AJ422028
P. alternans Lindenb. & Gottsche	Costa Rica	*Heinrichs et al. 4317*	AJ422029
P. amicta Steph.	Costa Rica	*Heinrichs et al. 4178*	AJ422022
P. bidens Gottsche	Brazil	*Gradstein 5378 (G)*	AF539458
P. bifaria (Sw.) Lindenb. var. *bifaria*	Tenerife	*Drehwald 3922*	AJ413173
P. bifaria var. *bifaria*	British Isles	*Rycroft 01014*	AY453387
P. bifaria var. *bifaria*	Brazil	*Costa & Gradstein 3805*	AY453388
P. bifaria var. *bifaria*	Costa Rica	*Heinrichs et al. 4394*	AY453389
P. bifaria var. *bifaria*	Ecuador	*Holz EC-01-113*	AJ422011
P. bifaria var. *bifaria*	Ecuador	*Holz EC-01-416*	AJ422010
P. bifaria var. *bifaria*	Bolivia	*Heinrichs et al. 4402*	AY453390
P. bifaria var. *bifaria*	Bolivia	*Heinrichs et al. 4069*	**AJ620673**
P. bifaria var. *bifaria*	Bolivia	*Heinrichs et al. 4076*	**AJ620674**
P. bifaria var. *rosea* (R.M.Schust.) J.Heinrichs	Venezuela	*Pócs et al. 9714/K*	**AJ620672**
P. buchtiniana Steph.	Bolivia	*Groth s.n.*	AJ413306
P. cristata (Sw.) Lindenb.	Costa Rica	*Heinrichs et al. 4192*	AJ422015
P. cucullifolia Jack & Steph. var. *cucullifolia*	Costa Rica	*Heinrichs et al. 4402*	AJ422012
P. cucullifolia var. *cucullifolia*	Ecuador	*Schmidt-Lebuhn 384*	**AJ620671**
P. cucullifolia var. anomala J.Heinrichs & S.Gradst.	Ecuador	*Holz EC-01-558*	AY330711
P. deflexa Mont. & Gottsche	Costa Rica	*Heinrichs et al. 4160*	AJ416083
P. deflexirama Taylor	Costa Rica	*Heinrichs et al. 4163*	AJ413310
P. dimorpha Lindenb. & Gottsche var. *ecuadorica* (Inoue) J.Heinrichs	Costa Rica	*Holz CR-00-499*	AJ422013
P. fuscolutea Taylor	Costa Rica	*Heinrichs et al. 4400*	AJ416086
P. longispina Lindenb. & Gottsche	Costa Rica	*Heinrichs et al. 4148*	AJ413307
P. ovata Lindenb. & Gottsche	Costa Rica	*Heinrichs et al. 4158*	AJ422017
P. patzschkei Steph.	Ecuador	*Holz EC-01-389*	AJ422018
P. punctata (Taylor) Taylor	British Isles	*Rycroft 01013*	AJ413174
P. retrorsa Gottsche	Costa Rica	*Heinrichs & al. 4154*	AJ422021
P. rutilans Lindenb	Bolivia	*Groth 101*	AJ416081
P. spinulosa (Dicks.) Dumort.	British Isles	*Rycroft 01012*	AJ413175
P. spinulosa	Belgium	*Dauphin et al. 3811*	AY275173
P. stricta Lindenb.	Ecuador	*Holz EC-01-478*	AJ416647
P. stricta	Costa Rica	*Heinrichs et al. 4401*	AJ416646
P. stricta	Tenerife	*Drehwald 3920*	AJ416649
P. stricta	Tenerife	*Rycroft 01071*	AJ416648
P. tocarema Gottsche	Costa Rica	*Heinrichs et al. CR199*	AJ413309
P. vincentina Lindenb.	Costa Rica	*Heinrichs et al. 4331*	AY275175
Plagiochilion mayebarae S.Hatt.	Japan	*Ohnishi 5588* (HIRO)	AY438238

the model selected by the hierarchical LRT was the TrN model (Tamura and Nei 1993) with gamma shape parameter (G) for among-site variation calculated from the data set (TrN + G). A ML analysis (with the TrN + G model) was implemented as a heuristic search with 10 random-addition-sequence replicates. The confidence of branching was assessed with 200 bootstrap resamplings in ML analysis (Felsenstein 1985; Hillis and Bull 1993).

MP analyses: Analyses were performed with the following options implemented: heuristic search mode with 10 random-addition-sequence replicates, tree bisection-reconnection branch swapping (TBR), MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Bootstrap support was estimated with full heuristic searcher, 1000 bootstrap replicates, and 10 random-addition-sequence replicates per bootstrap replicate, TBR swapping, MULtrees option on, collapse zero-branches off, and by saving all trees.

Morphological investigation

The morphological investigation was based on an isotype of *Rhodoplagiochila* (TNS), the new *Rhodoplagiochila*

vouchers (GOET, EGR), and numerous specimens of *Plagiochila* sect. *Arrectae* from the herbaria F, FLAS, G, GOET, INB, JE, LPB, MO, NY, RB, S, STR, U, W.

Results

Molecular investigation

ML analysis

The ML analysis produced a single tree (Fig. 1) in which the 38 ingroup sequences are placed in eight robust sectional clades. Only a few deeper nodes have good bootstrap support. *Plagiochila* sect. *Alternantes* Carl is sister to the remainder of the genus; but this topology is not supported. An unsupported clade comprises representatives of *P.* sects. *Adiantoideae* Lindenb., *Fuscoluteae* Carl, *Glaucescentes* Carl, *Hylacoetes* Carl, and *Vagae* Lindenb. Within this clade, the *Adiantoideae*, *Fuscoluteae*, and *Hylacoetes* form a well-supported monophyletic lineage. The above clade is placed sister to a clade with *P.* sects. *Arrectae* and *P.* sects. *Rutilantes* Carl, in a robust sister group relationship (BS of 90).

Samples of *Plagiochila cucullifolia* J.B.Jack & Steph. (*Szweykowskia*) from Costa Rica and Ecuador form a well-supported monophyletic lineage within *P.* sect. *Hylacoetes*. *Plagiochila cucullifolia* var. *anomala* J.Heinrichs & Gradst. from Ecuador is sister to var. *cucullifolia* with good support. *Plagiochila dimorpha* var. *ecuadorica* (Inoue) J.Heinrichs (*Steereochila*) is revealed as a further member of the *Hylacoetes* section.

The *Arrectae* clade is made up of sequences of *Plagiochila bidens* Gottsche, *P. retrorsa* Gottsche, *P. punctata* (Taylor) Taylor, *P. patzschkei* Steph., *P. spinulosa* (Dicks.) Dumort. (two sequences in a robust monophyletic lineage), and *P. stricta* Lindenb. (four sequences in a weakly supported monophyletic lineage). Nine samples of *P. bifaria* (Sw.) Lindenb. from Macaronesia, the British Isles, Brazil, Costa Rica, Ecuador and Bolivia and *R. rosea* from Venezuela form a robust clade (BS of 94) within *P.* sect. *Arrectae*. *Rhodoplagiochila rosea* is sister to the *P. bifaria* clade. This position is not well bootstrap-supported. Eighty-four (ITS1: 50; 5.8S: 1; ITS2: 33) of the 729 aligned positions of the sequences related to the *P. bifaria*/*Rhodoplagiochila* clade are variable, 22 (ITS1: 15; ITS2: 7) as a result of deletion/insertion events.

MP analysis

Of the 729 investigated characters, 205 were parsimony informative, 126 autapomorphic, and 398 constant. The heuristic search recovered 43 equally most parsimonious trees (not shown) with a length of 723 steps, a consistency index (CI) of 0.62, a CI excluding uninformative characters of 0.49, a retention index (RI) of 0.75, and a rescaled consistency index of 0.46. The strict consensus of these trees (Fig. 2) is largely congruent with the ML tree (Fig. 1). Differences are found only in unsupported or weakly supported topologies. Contrary to the ML topology, *P.* sect. *Vagae* is placed sister to the remainder of *Plagiochila*. *Plagiochila* sect. *Alternantes* and *P.* sect. *Glaucescentes* form an unsupported sister relationship and are placed sister to a clade containing the robust *Adiantoideae–Fuscoluteae–Hylacoetes* and *Arrectae–Rutilantes* clades. The nine *P. bifaria* sequences plus *Rhodoplagiochila* form a well-supported clade within *P.* sect. *Arrectae*. The *Rhodoplagiochila* sequence and the three *P. bifaria* sequences from Bolivia form a polytomy at the base of the *P. bifaria* clade.

Morphology

The isotype of *R. rosea* is in good accordance with the detailed description of Schuster (2000), except for the leaf surface which is densely beset with small, low, globose to ellipsoidal papillae. The new *Rhodoplagiochila* voucher material from which DNA was extracted is very similar in all respects (see Fig. 3). Leaves with papillose border areas have occasionally been observed within *P. bifaria* (Heinrichs et al. 1998a), but completely rough leaves were so far unknown from this taxon. Another difference is found in the leaf margin which is ciliately toothed all-around in *Rhodoplagiochila*. *Plagiochila bifaria* usually has an entire dorsal leaf margin (e.g., Heinrichs et al. 1998a, 2004). No plants with rose-red shoot apices were detected in the dry isotype of *Rhodoplagiochila*, nor in the DNA voucher *Pócs* et al. *9714/K* or the specimen *Pócs 9721/AP*. The tiny plants had a brownish to weakly rusty red-brown pigmentation similar to that of other high Andean phenotypes of *P. bifaria*. According to field observations of Pócs, the plants were rose-red when fresh.

As an outcome of the molecular and the morphological investigations, *R. rosea* is here treated as a variety of *P. bifaria*.

***Plagiochila bifaria* (Sw.) Lindenb. var. *rosea* (R.M.Schust.) J.Heinrichs, comb. et stat. nov.**

R. rosea R.M.Schust., Phytologia 39: 247, 1978.

Type: Venezuela, Mérida. Sierra do Santo Domingo between Lagunita Verde and Laguna los Patos, 3700–3750 m, *Schuster & Ruiz-Teran 76-901* (holotype, herb. Schuster, not available upon request; isotype, TNS!).

Additional specimens examined: Venezuela, Mérida, P.N. Sierra Nevada, Estación Loma Redonda, Los Nevados, 4100 m, 1997, *Pócs, Frahm & León 9714/K* (GOET, EGR, MERC); Estación La Agunda, subpáramo, 3250 m, *Pócs 9721/AP* (EGR).

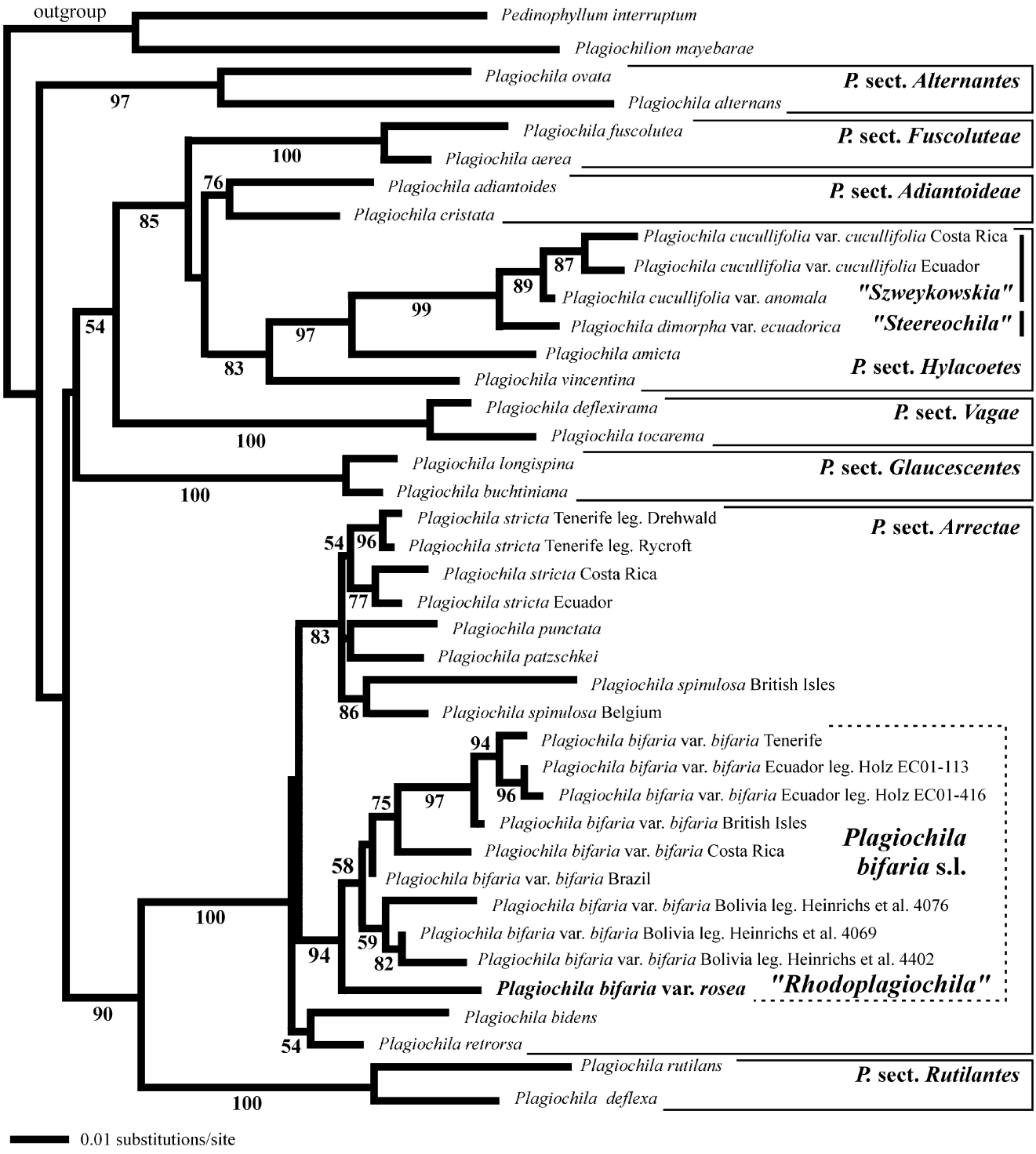
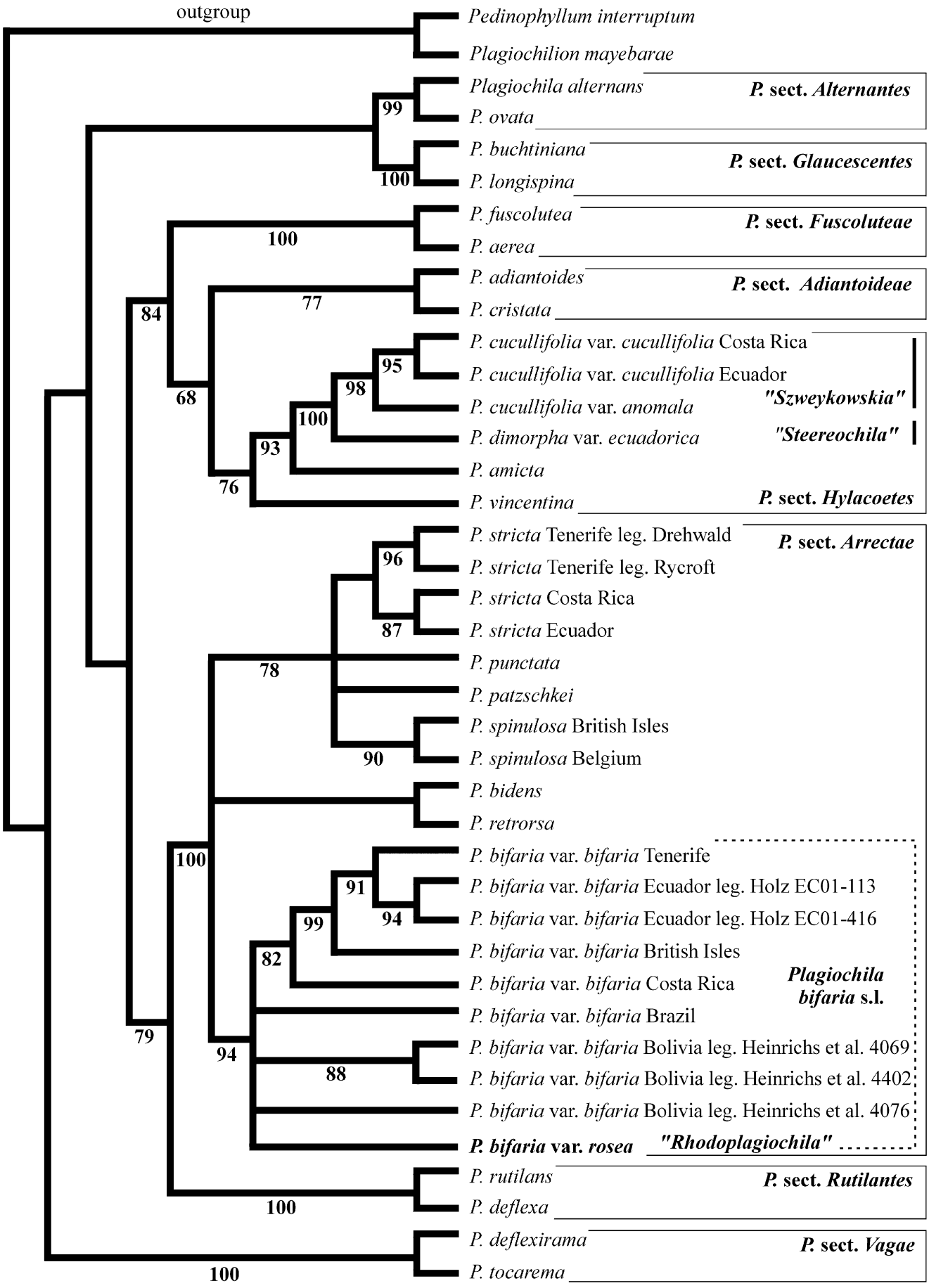


Fig. 1. Molecular phylogeny of *Plagiochila* species based on nrDNA ITS1, 5.8S, and ITS2 sequence comparisons using 729 aligned positions. The rooted tree resulted from a ML analysis of 40 sequences (including the outgroups), using the TrN + G model with estimated gamma shape ($G = 0.53$), calculated as the best model by Modeltest 3.06 (Posada and Crandall 1998); bootstrap percentage values (> 50%) were determined for ML (using TrN + G).

P. bifaria is widespread in mountainous, humid regions of the Neotropics and in Atlantic Europe. It occurs at altitudes between 500 and 4200 m (Heinrichs et al. 2004), most frequently as an epiphyte. The new variety, however, has been collected only thrice in the high Andes of Mérida, Venezuela, growing on soil in páramo or on bark in cloud forest vegetation between 3250 and 4100 m.


outgroup
Pedinophyllum interruptum
Plagiochilion mayebarae
Plagiochila alternans
P. ovata
P. sect. Alternantes
P. buchtiniana
P. longispina
P. sect. Glaucescentes
P. fuscolutea
P. aerea
P. sect. Fuscoluteae
P. adiantoides
P. cristata
P. sect. Adiantoideae
P. cucullifolia var. cucullifolia Costa Rica
P. cucullifolia var. cucullifolia Ecuador
P. cucullifolia var. anomala
"Szweykowskia"
P. dimorpha var. ecuadorica
"Steereochila"
P. amicta
P. vincentina
P. sect. Hylacoetes
P. stricta Tenerife leg. Drehwald
P. stricta Tenerife leg. Rycroft
P. stricta Costa Rica
P. stricta Ecuador
P. sect. Arrectae
P. punctata
P. patzschkei
P. spinulosa British Isles
P. spinulosa Belgium
P. bidens
P. retrorsa
P. bifaria var. bifaria Tenerife
P. bifaria var. bifaria Ecuador leg. Holz EC01-113
P. bifaria var. bifaria Ecuador leg. Holz EC01-416
P. bifaria var. bifaria British Isles
P. bifaria var. bifaria Costa Rica
P. bifaria var. bifaria Brazil
P. bifaria var. bifaria Bolivia leg. Heinrichs et al. 4069
P. bifaria var. bifaria Bolivia leg. Heinrichs et al. 4402
P. bifaria var. bifaria Bolivia leg. Heinrichs et al. 4076
P. bifaria var. rosea
"Rhodoplagiochila"
Plagiochila bifaria s.l.
P. rutilans
P. deflexa
P. sect. Rutilantes
P. deflexirama
P. tocarema
P. sect. Vagae
99
100
100
84
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88
100
100

Key to the varieties of *P. bifaria* (see also Fig. 3)

– Leaf margin toothed all-around, apical teeth of leaf ciliate; cuticle rough. . . . . *P. bifaria var. rosea*
– Dorsal leaf margin usually entire, apical teeth of leaf triangular to elongate triangular (rarely ciliate); cuticle smooth or border areas of leaves slightly rough. . . . . *P. bifaria var. bifaria*

Discussion

Until very recently, the systematic study of bryophytes has been conducted by analysis of morphological characters. However, their limited number and their often unclear taxonomic value have led to numerous conflicting hypotheses. On the other hand, the study of variable molecular markers such as nrITS has proven to be a powerful tool for the testing of morphological concepts, as well as for inferences on phylogenetic relationships from species to family level (e.g., Baldwin et al. 1995; Soltis and Soltis 1998; Hershkowitz et al. 1999).

The tree topology derived from the above ITS alignment clearly supports Schuster's opinion that *Rhodoplagiochila* belongs to Plagiochilaceae. However, separate generic status of *Rhodoplagiochila* is not confirmed. As already assumed by Heinrichs (2002), *Rhodoplagiochila* is a member of the Neotropical–Atlantic European *Plagiochila* sect. *Arrectae*. This group comprises small to medium sized species characterized by nearly exclusively intercalary branching, obliquely spreading to laterally oppressed leaves often provided with a vitta, a small-meshed leaf cell pattern, an often rough cuticle, simple, intercalary androecia, cylindrical perianths covered partly by bracts, a capsule wall with thickenings in all layers, unicellular, baculate spores, and normally unispiral, rough elaters (e.g., Heinrichs 2002; Groth et al. 2003). The somewhat reddish colour of the fresh plants of the *Rhodoplagiochila* holotype described by Schuster (1978, 2000) is also known from high Andean samples of *P. bifaria* with rusty red-brown pigmentation, represented in the molecular tree by samples *Heinrichs* et al. *4069* and *4402* from Bolivia.

The morphology, phytochemistry and distribution of *P. bifaria* has been the subject of several recent papers (Heinrichs et al. 1998a, b, 2004; Rycroft et al. 1999; Rycroft 2003). Heinrichs et al. (2004) discussed the phenotypic and phytochemical variation of *P. bifaria* and demonstrated that the extreme morphological variation is not reflected in ITS topologies. Even though a large number of variable positions is found in the *P. bifaria* ITS sequences, a rather diffuse phylogenetic signal is derived from the alignment. Accordingly, Heinrichs et al. (2004) abstained from an infraspecific classification of *P. bifaria* with several intergrading extreme morphotypes differing in size, dentition, and ramification. The new *P. bifaria* variety, however, can be separated by differences in two widely stable morphological characters (leaf surface and presence of teeth on ventral margin). It is also placed sister to the remaining *P. bifaria* genotypes in the ML tree, although bootstrap support for this is only weak. Infraspecific variation in presence/absence of papillae is also accepted for other species of *P.* sect. *Arrectae*, e.g., *P. punctata* or *P. spinulosa* (Paton 1999). Wide fluctuation in the dentition of leaves and perianths has been reported from numerous *Plagiochila* species (e.g., So 2001; Heinrichs 2002).

Morphological differentiation of genetically heterogenous entities is not a general phenomenon in bryophytes. McDaniel and Shaw (2003) demonstrated that the morphologically only weakly differentiated subspecies of *Pyrrhobryum mnioides* (Hook.) Manuel differ genetically more than many genera of pleurocarpous mosses. Longton and Hedderson (2000) pointed out high genetic distances within the morphologically rather uniform moss taxon *Bryum argenteum* Hedw.

Although occasionally tested by molecular phylogenetic analyses, species concepts in bryology are still essentially based on morphology. *Plagiochila* taxonomy has been regarded as "daunting, forbidding, confusing and notoriously difficult" (So 2001), most likely because of the paucity of stable morphological characters and the high infraspecific variation. Considering the difficulties with justifying species boundaries in *Plagiochila*, previous morphological concepts are in remarkably good accordance with topologies derived from nrITS analyses (e.g., Heinrichs 2002; Renker et al. 2002; Rycroft et al. 2002; Groth et al. 2003). In other bryophyte groups conflicts arose between molecular trees and morphological concepts. Shaw and Allen (2000) demonstrated that morphospecies of *Fontinalis* are non-monophyletic in phylogenetic trees derived from cp and nrDNA sequences. Vanderpoorten et al. (2001) arrived at the same conclusion for *Hygroamblystegium tenax*, using nrITS sequences. Shaw (2000) concluded that *Mielichhoferia mielichhoferiana* is paraphyletic in ITS topologies.

In the Neotropics, *P. bifaria* and other representatives of the *Arrectae* are restricted to mountainous regions. The greatest morphological and chemical variation within *P. bifaria* is found in the northern and central

Fig. 2. Rooted strict consensus tree of 43 equally parsimonious trees recovered during 10 random taxon addition heuristic searches of the ITS data set. Bootstrap support (> 50%) is indicated at branches.

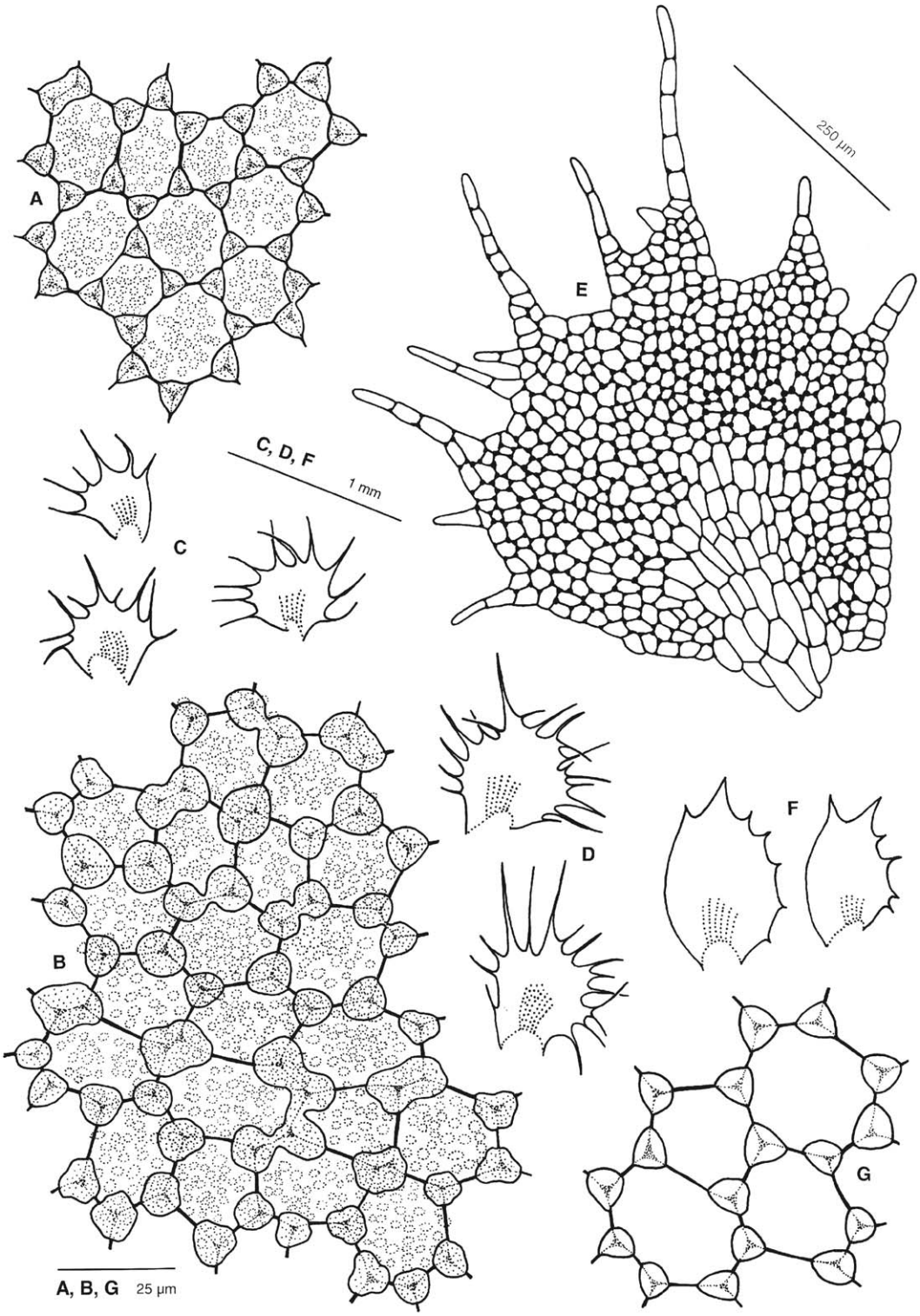

Fig. 3. Leaf morphology of *Plagiochila bifaria* var. *rosea* (R.M.Schust.) J.Heinrichs (A–E) and *P. bifaria* var. *bifaria* (F,G). (A,B) Cells from upper half of leaf, papillae of leaf surface dotted; (C,D,F) leaf outlines, vitta dotted. (E) leaf, cellular; (F) cells from upper half of leaf, surface smooth; (A,C) from isotype of *Rhodoplagiochila rosea* (TNS); (B,D,E) from *Pócs* et al. *9714/K* (GOET); and (F,G) from isotype of *P. bifaria* (G19805).

Andes (southwards to Central Bolivia) and in the high mountains of Central America where the species occupies a wide range of humid habitats, ranging from cloud forest to páramo (Heinrichs et al. 2004). These habitats are the product of geological and climatic changes which originate from Late Miocene uplift. Prior

to this uplift event the region was presumably dry (Parrish 1990) and not occupied by the moist forests which are now populated by *P. bifaria*. The emergence of numerous niches for plants adapted to humid conditions may have led to rapid morphological diversification. Observations within an Andean species group of *Plagiochila* sect. *Hylacoetes*, including the former genera *Steereochila* and *Szweykowskia*, point in the same direction. Although gametophytes in this group differ in numerous characters (in particular leaf shape, leaf orientation and dentition), ITS variation is low (Heinrichs et al. 2003). Evidence for a sudden diversification of *P. bifaria* and other members of *P.* sect. *Arrectae* is also found in the ML tree topology (Fig. 1) with short branch lengths within the section. In contrast, the most recent common ancestor of the extant *Arrectae* species has a long branch. The topology is most likely not the result of incomplete taxon sampling, since a stable sister relationship between the *Arrectae* and *Rutilantes* as well as similar branch lengths were also found in analyses of larger data sets (e.g., Groth et al. 2003).

Until recently, tropical America has been regarded a centre of diversity of the Plagiochilaceae (Gradstein and Reiner-Drehwald 1995). However, our results indicate that in the Neotropics Plagiochilaceae in the circumscription of Crandall-Stotler and Stotler (2000) are represented solely by *Plagiochila*. *Steereochila*, *Szweykowskia*, and *Rhodoplagiochila* are clearly subordinate parts of this genus. In contrast, representatives of the Plagiochilaceae genera *Chiastocaulon*, *Pedinophyllum*, and *Plagiochilion* are placed sister to *Plagiochila* using nrITS as well as cp *rps*4 sequences (Groth and Heinrichs 2003), which points to a centre of diversity of the Plagiochilaceae in southeastern Asia and Australasia.

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